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The application of Cyanoacrylate-based Filling Material for Surgically Created 1-wall Intrabony Defects in Dogs

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Bone grafts are often used as part of a surgical protocol to regenerate periodontal structures. Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) are used as bone graft materials for periodontal regeneration. N-butyl-2-cyanoacrylate is also used as a tissue adhesive because of its rapid adhesion to hard and soft tissue. We evaluated the bioactive properties of HA/β-TCP and cyanoacrylate filling materials for clinical applications. In four male beagle dogs, we bilaterally created 4×4 mm one-wall intrabony defects at the distal aspect of the second mandibular premolars, and at the mesial aspect of the fourth mandibular premolars. These defects were either experimentally treated with a HA/β-TCP and cyanoacrylate combination, cyanoacrylate only, or surgery with no filling material. The dogs were killed eight weeks after surgery, and block sections of the defects were collected for histologic and histometric analysis. Postoperative healing was uneventful. Histological analysis revealed no significant differences in periodontal healing between the experimental sites that received grafted materials and those that did not. Only small amounts of bone fill and cementum regeneration were observed. In this study, we expected that the osteogenic filling material would become fixed in the bony defect more quickly and stably because of the adhesive ability of cyanoacrylate; however, the combination filling material did not result in a higher amount of new bone and cementum formation compared to surgery with no filling material.

Key words: bone graft, cyanoacrylate, hydroxyapatite, β- tricalcium phosphate

Introduction

The objective of periodontal therapy is to regenerate the periodontal attachment including cementum, periodontal ligament, and alveolar bone. Bone grafting is used as a component of surgical protocols aimed at regenerating periodontal structures. Bone grafting success relies not only on osteogenic potential of the grafting material, but also on the stability and space maintenance of the grafted materials. The biomaterials hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have structures and compositions that are similar to the inorganic constituents of bone, and are thus often used as bone graft material.[1,2)]

N-butyl-2-cyanoacrylate has been used as a tissue adhesive and has a wide range of applications in surgery because of its rapid adhesion to hard and soft tissue.[3,4)] In 2005, Park et al. investigated the favorable physical and bioactive properties of cyanoacrylate-based filling materials in some depth.[5)] The objective of this study was to evaluate the bioactive properties of cyanoacrylate-based filling materials composed of HA/β-TCP and cyanoacrylate for clinical applications.

Materials and Methods

Animals

Four male beagle dogs(18-24months) were used. The dogs had intact dentition and healthy periodontiums. The selection of animals, management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

Materials

Graft materials- consisted of 70% HA, 30% β-TCP (Osteon Dentium, Seoul, Korea), and N-butyl-2-cyanoacrylate (YESBIO., Seoul, Korea).

Experimental groups

There were three experimental groups (1) control group : flap only, (2) HA/β-TCP and cyanoacrylate combination, and (3) cyanoacrylate only

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Surgical protocols

Teeth were extracted under general anesthesia using 0.05 mg/kg atrophine subcutaneous, 2 mg/kg xylazine (Rompun; Bayer Korea, Seoul, Korea), and 10 mg/kg ketamine hydrochloride (Ketalar; Yuhan Company, Seoul, Korea) intravenous. The dogs were placed on a heating pad, intubated, and administered 2% enflurane, and 2% lidocaine HCl with 1:100,000 epinephrine (KwangMyung Pharmaceuticals, Seoul, Korea) by infiltration at the surgical site. The first and third mandibular premolars were carefully extracted prior to the experimental surgery. Flaps were sutured with 5-0 resorbable suture material (Polyglactin 910, braided absorbable suture, Ethicon; Johnson & Johnson International, Edinburgh, UK). On the day of the surgery, the dogs received 10 mg/kg IV Cefazoline antibiotic. After a healing period of eight weeks, the mandibular mucoperiosteal flaps were elevated, and 4 × 4 mm one-wall intrabony defects were surgically created bilaterally at the distal aspect of the second premolars and, at the mesial aspect of the fourth premolars. A reference notch was made with a round bur on the root surface at the base of each defect. The defects were then experimentally treated with a combination of HA/β-TCP and cyanoacrylate, cyanoacrylate only, or a flap only (Figure 1). The HA/β-TCP and cyanoacrylate combination filling material was prepared by mixing cyanoacrylate and acid-treated HA particles. The dogs were killed eight weeks after surgery, and block sections of the defects were collected for histologic and histometric analysis. The central sections from each block were stained with hematoxylin and eosin (H&E stain) and examined using light microscopy.

Histologic and histometric analysis

We used a computer-based image analysis system (Image-pro-Plus; Media Cybernetics, Silver Spring, MD, USA) to observe the experimental sites with regard to junctional epithelium migration, connective tissue regeneration, new bone and cementum formation, root resorption, ankylosis, and the state of the grafted materials. For the histometric analysis, the cementoenamel junction (CEJ) and the notch were used as reference points. The histometric parameters were: (1) defect height- the distance from the CEJ to the base of the reference notch; (2) junctional epithelium migration- the distance from the CEJ to the apical extension of the junctional epithelium; (3) connective tissue adhesion- the distance from the apical extension of the junctional epithelium to the coronal extension of cementum regeneration; (4) cementum regeneration-the distance from the base of the reference notch to the coronal extension of the newly formed cementum on the root surface; (5) alveolar bone regeneration- the distance from the base of the reference notch to the coronal extension of the newly formed alveolar bone along the root surface.

Figure 1. Clinical photograph of 1-wall intrabony defects. Cyanoacrylate and HA/β-TCP combination was grafted at the defects.

Statistical analysis

We used histometric recordings from sections in each block to calculate the mean scores and standard deviation (mean ± SD). The mean scores were used to test for differences among the experimental conditions using ANOVA and the post hoc *t*-test was used for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results and Discussion

The regeneration after periodontal treatment means the regain of lost supporting tissues, including new cementum, new periodontal ligaments and new alveolar bone. Currently, however, complete and predictable true regeneration is difficult to attain. There have been various attempts to accomplish true periodontal regeneration, and several investigations have demonstrated that bone grafts clinically result in new attachment and bone formation.[6] Of the various bone graft materials, autogenous bone is regarded as the gold standard, because of its osteogenic potential and biocompatibility.[7] However, autobone graft is limited by the available volume, the need for another surgery site, and risk of external root resorption and ankylosis. To overcome these disadvantages, synthetic bone grafts have been proposed for periodontal treatment. The structure and composition of HA and β-TCP are similar to the inorganic constituents of bone, leading to their use as bone graft material. Many studies have yielded favorable results with biphasic calcium phosphate (BCP), which combines the scaffold properties of HA and the resorbality of β-TCP. A combination of HA and β-TCP leads to more new bone formation than when HA is used alone.[8]

Saska et al. (2009) showed that fixation with the adhesive N-butyl-2-cyanoacrylate led to a significantly greater area of bone graft than screw fixation.[3] In this study, we added the combination of HA and β-TCP to cyanoacrylate to improve the bioactivity and stabilization of the graft particles. Our objective was to

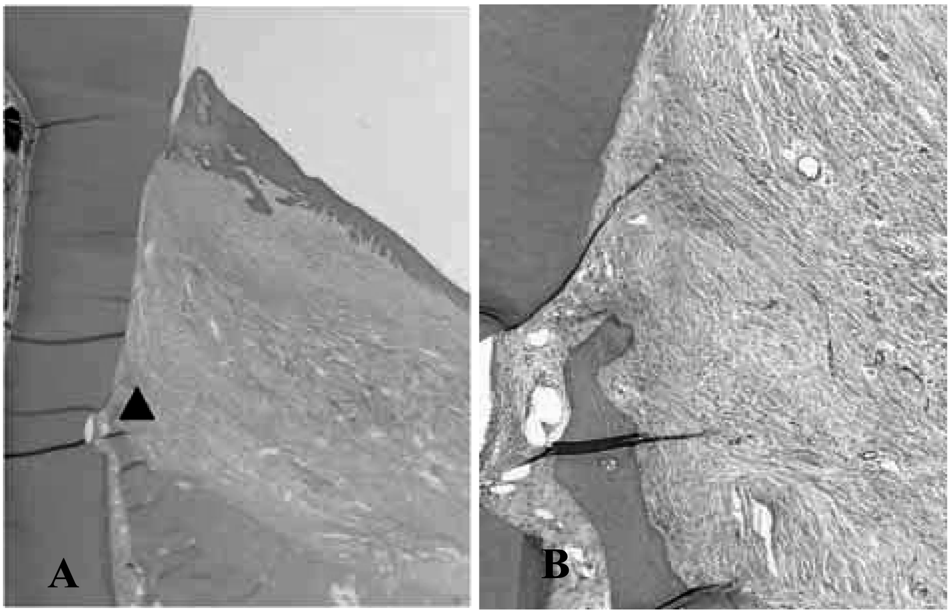

Figure 2. Histologic view of control (flap-only) experimental site. Defect margin and new bone are indicated by the arrowhead. A small amount of bone fill and cementum regeneration can be seen. H&E stain, 10× magnification (A), 40× maginification (B).

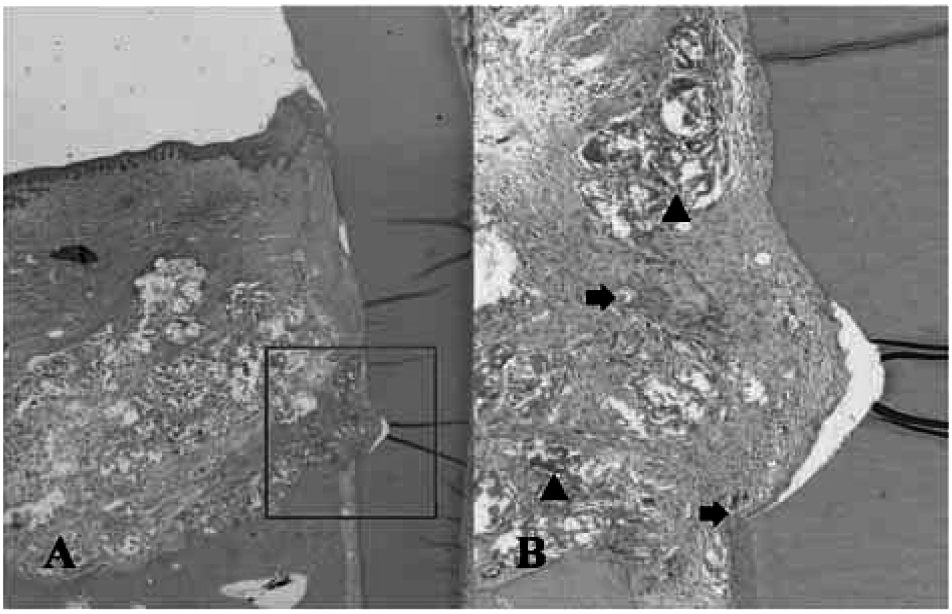

Figure 3. Histologic view of cyanoacrylate and HA/β-TCP combination experimental site. Arrow; new bone area. Arrow-head; residual graft materials. H&E stain, 10× magnification (A), 40× maginification (B).

evaluate the biological properties of cyanoacrylate-based filling materials comprising HA and β-TCP for dental applications.

Clinically, wound healing was uneventful. There were no infections and no complications during the experiment period.

Histological analysis did not reveal a significant difference in periodontal healing among the sites that received graft materials and those that underwent the control, flap-only surgery. In the control group, we observed only a small amount of bone fill and cementum regeneration. Epithelial migration and connective tissue ingrowth were also observed. The periodontal ligament fibers were generally oriented in a direction parallel to the root surface in the suprabony area. The defect was filled with dense fibrous connective tissues and minimal inflammatory cell infiltration such as neutrophils and lymphocytes was observed at surgical sites (Figure 2). The experimetal group had histologic results similar to those of the control group. The HA/β-TCP and cyanoacrylate combination group showed a small amount of new bone formation and

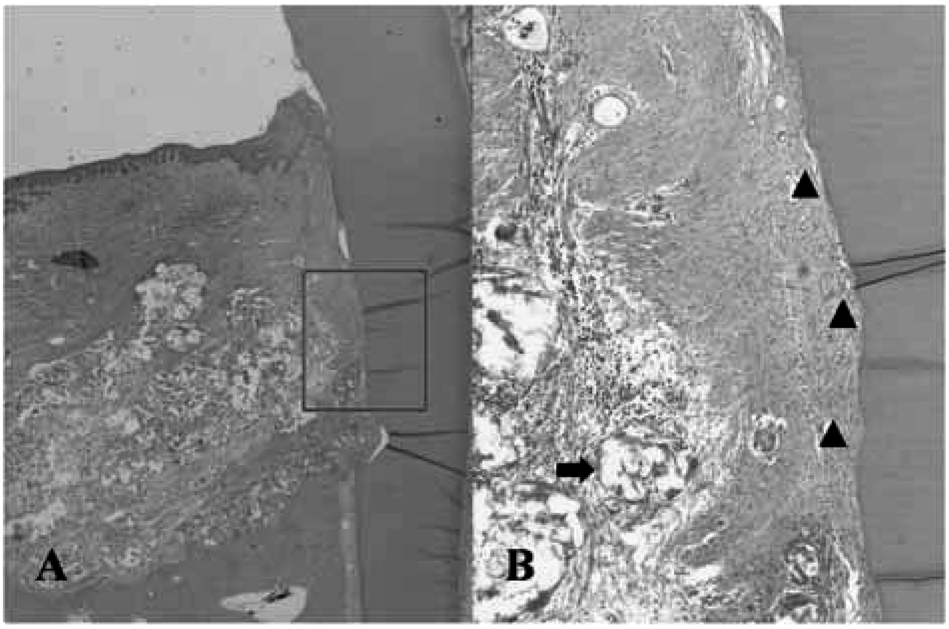

Figure 4. Histologic view of cyanoacrylate-only experimental site. H&E stain, 10× magnification (A), 40× magnification (B) Arrow: fatty marrow, Arrow-head: osteoblast lining.

Table 1. Histometric analysis (mean ± standard deviation, mm, N = 4)

	Cyanoacrylate +HA/β-TCP	Cyanoacrylate only	Control
Defect height	4.46 ± 0.41	4.36 ± 0.27	4.09 ± 0.18
Bone regeneration	0.25 ± 0.31	0.37 ± 0.58	0.23 ± 0.06
Cementum regeneration	2.06 ± 0.70	1.80 ± 0.71	2.55 ± 0.52
Epithelial migration	2.20 ± 1.05	1.74 ± 0.89	1.48 ± 0.74
Connective tissue adhesion	0.16 ± 0.08	0.17 ± 0.12	0.07 ± 0.05

epithelial migration. This new bone formation was originated from apical aspect of defects in control and HA/β-TCP and cyanoacrylate combination group.[9)]

In some parts, osteoblast and osteoids were observed around the bone marrow. The lamellar bone could be distinguished and we observed periodontal ligament fibers between new bone and new cementum. Residual grafted materials were observed at the connective tissue area (Figure 3-A and 3-B). In the cyanoacrylate-only group, the amount of newly formed bone and cementum was similar to that of the other groups. However, we observed some inflammatory cells in the connective tissue region and near the junctional epithelium. Loose connective tissue and a few pores were observed in the defect area (Figure 4-A and 4-B). This suggests that some physical characteristic of cyanoacrylate disturbs natural healing of the defect and induces an inflammation reaction, in contrast to what we observed in the control group.

The results from our histometric evaluation are presented in Table 1. The histometric results were similar to those from the histologic evaluation in that there were no statistically significant differences among the groups. The amount of new alveolar bone was an average of 0.25 ± 0.31 mm, 0.37 ± 0.58 mm, and 0.23 ± 0.06 mm for the HA/β-TCP and cyanoacrylate group, the cyanoacrylate-only group and the flap-only group, respectively. The new cemetum formation was 2.06 ± 0.70 mm, 1.80 ± 0.71 mm, and 2.55 ± 0.52 mm for the HA/β-TCP and cyanoacrylate group, the cyanoacrylate-only group and in flap-only group, respectively. We had expected that combining HA/β-TCP and cyanoacrylate would improve the ability of the filling material to maintain the space and would be easier to manipulate; consequently, the combination would promote the formation of new bone and cementum more effectively than the cyanoacrylate-only or the flap-only group. However, we could not discern any differences in bone and cementum formation among the groups. This could be due to the physical properties of cyanoacrylate, which may inhibit angiogenesis for new bone formation and could be slightly cytotoxic. Based on our results, we believe that further research is required to overcome the limitations of cyanoacrylate-based filling materials for dental applications.

Conclusion

In this study, we expected that osteogenic filling materials would be fixed in bony defects more quickly and stably, because of the adhesiveness of the cyanoacrylate. However, neither the combination of cyanoacrylate and HA/β-TCP or cyanoacrylate-only yield osteogenic results superior to the flap-only control group.

Acknowledgements

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